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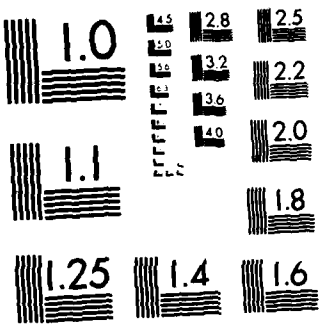

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DEVELOPMENT OF PROPHYLACTIC ANTI-FUNGAL PREPARATIONS

Final Report

By
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In order to develop better topical anti-fungal agents with prophylactic activity against common ringworm infection a chemical assay for sodium pyrithione (a known anti-fungal drug) was developed in stratum corneum and its persistence there determined a dose response study. To evaluate its effectiveness clinically a double blind trial was carried out utilizing human volunteers and experimental model for superficial fungus infection of skin. The results showed that sodium pyrithione persisted in an effective concentration in stratum corneum for 3 days after stopping application and it was concluded that an 0.5% lotion of sodium pyrithione warrants serious

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consideration as an alternative to Griseofulvin for prophylactic use when and if needed to combat superficial ringworm infections.

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FOREWORD

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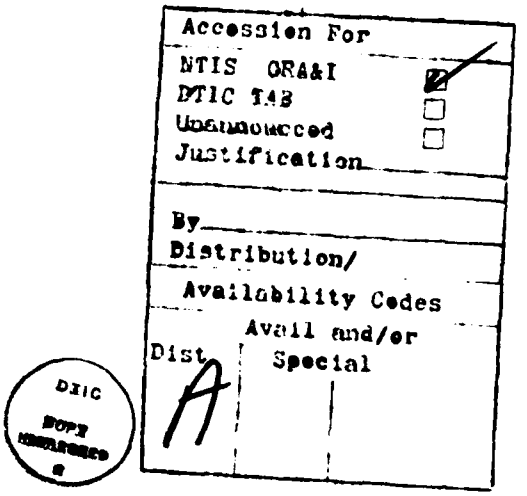

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FINAL PROGRESS REPORT

FOR

Contract No. DAMD17-75-C-5003

Description of Project

The long-range goal of this research project is to identify effective topical anti-fungal agents with prophylactic activity against trichophyton metagrophytes (ringworm infections) prevalent in the U.S. Army personnel exposed to tropical swamps. The candidate compounds are to be screened in an appropriate formulation for their potential topical prophylactic anti-fungal activity utilizing the controlled infectivity test method developed by Allen, Reinhardt, Ackers, Gunnison and co-workers (1,2). Earlier reports summarized studies on the activity of griseofulvin relative to clotrimazole, miconazole, and thiabendazole (3). This present report will direct its attention primarily to investigations of sodium pyrithione.

Specific Aims

1) To attempt to establish assays for sodium pyrithione and its chemical analogues with sufficient sensitivity and specificity to allow one to monitor their levels in milligram quantities of horny layer tissue.

2) If successful in establishing assays, evaluate the accumulation on repeated application and the rate of depletion on discontinuing the application of sodium pyrithione as made avialable in a lotion form on IND-8293 by G.S. Herbert Labs, Division of Allergan Pharmaceuticals, Irvine, California 92664.

3) To investigate whether sodium pyrithione possesses sufficient topical anti-fungal activity in controlled infectivity tests compared with griseofulvin to warrant further study.

4. To establish how long sodium pyrithione persists in the skin concentration sufficient to inhibit growth of T. Menta using the controlled infectivity test.

Background

Utilizing chemical and controlled clinical studies, investigations have been undertaken to assess the topical prophylactic activity of a number of drugs against T. Menta intections. These included griseofulvin, clotrimazole, miconazole and thiabendazole. Each of these compounds were applied to skin

sites up to a ten-day period. Skin scrapings were obtained 24 hours and 96 hours after discontinuing the drug. The data are summarized in Table I (3). The concentrations reported for griseofulvin in this table are approximately 45 fold the concentration obtained after the oral administration. The chemical persistence of these compounds in the skin up to 96 hours after application led us to investigate their topical prophylactic activity using the controlled clinical infectivity procedure of Allen et al (1,2).

A double blind trial was conducted to determine the efficacy of the topically applied anti-fungal agents preventing the infection of T. Menta spores under standard condition. The spores were prepared by Dr. Joseph Greenberg, a microbiologist. An aliquot containing 600 spores was used as the inoculum. The volunteers were montiored by Dr. William Epstein, who was unaware of the site of application of any of the chemicals. The chemical compounds were applied in the formulations listed in Table I in each of four areas. Each area was infected with 600 spores of T. Menta. The sites were occluded with saran wrap for four days and then were removed. The infection was scored weekly as follows: (0), +1 or +2. according to a predetermined definition of the scoring values.

Before breaking the code, a week was chosen on an individual basis on which the greatest degree of response occurred. The results of this experiment were statistically analyzed using a chi-square contingency table approach from which it was concluded that griseofulvin was significantly more effective as a prophylactic agent against T. Menta under the test procedure than was observed miconazole or clotrimazole (4). Therefore, these compounds are not sufficiently active to be proposed as an alternative to griseofulvin as a prophylactic compound against T. Menta. Separate studies comparing thiabendazole with griseofulvin led to the same degree of response as clotrimazole and miconazole and therefore it also was found to be insufficiently active to be a candidate in the prophylactic treatment against T. Menta.

Analytical Developments

Attention was therefore turned to the potential anti-fungal prophylactic activity of sodium pyrithione. A number of different chemical compounds have been suggested as potential metabolites of sodium pyrithione. These include the pyrithione disulfide, 2-mercaptopyridine, the pyridine disulfide and the pyrithione sulfonic acid. Each of these compounds were postulated as possibly occurring in skin scrapings due to metabolic processes occurring in the skin or chemical changes that could take place during storage extraction and/or sample processing. Extensive investigations of these compounds and their inter-convertibility was therefore undertaken.

The major compounds of interest are pyrithione, its reduced form-

TABLE I

Average epidermal concentrations of drug after 10 days topical application

Drug	Concentration in skin (mcg/mg) Mean ± S.D.	
	24 hours after discontinuing the drug	96 hours after discontinuing the drug
Miconazole, 2% cream	2.59 ± 1.67	0.142 ± 0.193
Clotrimazole, 1% in PEG*	1.99 ± 1.12	0.40 ± 0.08
Griseofulvin, 0.45% in alcohol*	11.71 ± 5.60	2.11 ± 0.99
Thiabendazole, 1% in alcohol*	37.01 ± 26.06	3.06 ± 2.17

*As previously reported in Annual Report 1975.

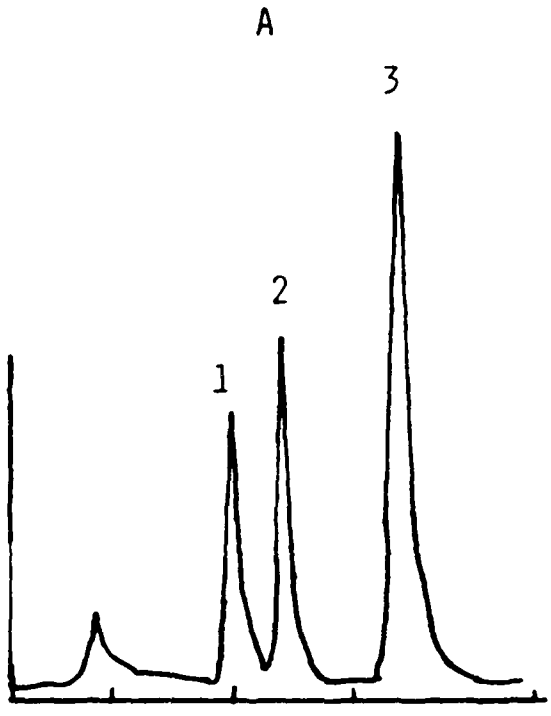
2-mercaptopyridine, and their disulfides:pyrithione disulfide and pyridine disulfide. An assay of each of these individual compounds were developed on an HPLC with a C-18-5 micron (Spherisorb) column using 62.5% methanol in 0.03 M-phosphate buffer pH 6.5 as the elution system-A for the assay of fresh solutions of the test compound excepting 2-mercaptopyridine using a 245 nm UV detection (Figure 1-A) and by using a 20% methanol-0.03M phosphate buffer 6.5 as elution system-B for 2-mercaptopyridine using 274 nm. (Figure 1-B). The use of two different chromatographic systems allows for better separation and a 6 fold increase in sensitivity. The system yielded linear response with a minimum detectable level of about 5 ng. (See Figure 2)

However, a problem of interconversion of the thiols and disulfides occurred when they were mixed in the organic sol its in which they were extracted and evaporated (N_2) dryness. On chror ography of mixtures of pyrithione disulfide with mercapto-pyridine or h pyridine disulfide or of mixtures of pyridine disulfide with pyrith ne, a large extraneous peak appears between those for the two disulfid aks (See Figure 3). This new peak is not seen when the thiols are m ith their respective disulfides. When the pyridine disulfide is mix ch pyrithione disulfide the extraneous peak appears and is accompanied with a diminution of the disulfide peaks. Therefore, it seemed likely that the new peak is a mixed disulfide with an intermediate redox state, e.g. half pyridine and half pyrithione. It is apparent that the oxidation reduction potentials of the compounds are sufficiently close to allow inter-conversion when mixed or when they occur together in the skin. Extraction of mixtures of the compounds from aqueous solution was followed by U.V. assay and it was once again verified that the compound readily inter-convert. An attempt was therefore made to reduce the compound to a common compound 2-mercaptopyridine using classical reducing systems e.g., stannous chloride, zinc dust, borohydride or cyanoborohydride. It was found that borohydride was useful in reducing the compounds. However, when blank skin extracts were spiked with these mixtures,spurious peaks occurred which suppressed our ability to quantitate the reaction. We believe these were due to an additional chemical reaction of the compounds with endogenous compounds in the horny layer.

Due to the complexity and lability of the system it was decided that a chemical assay was not feasible, therefore the analysis of sodium pyrithione and its chemical forms in the horny layer tissue samples was deemed beyond present technology and was dropped.

Dose Ranging Studies

A double-blind study was conducted in order to investigate what concentration of sodium pyrithione in the form of a topical lotion furnished by G.S. Herbert Laboratories would be effective in controlled activity tests. All subjects participating in infectivity studies were



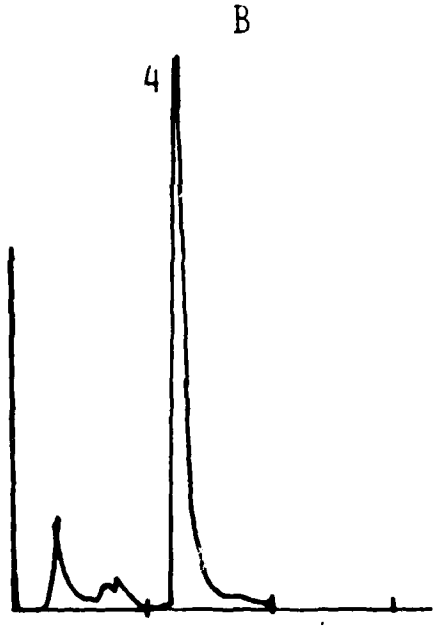


Fig 1-A: HPLC chromatograms of pyrithione disulfide (1),pyridine disulfide (2) and pyrithione (3) using elution system A.

Fig 1-B: HPLC chromatograms of 2-mercapto-pyridine (4) using elution system B.

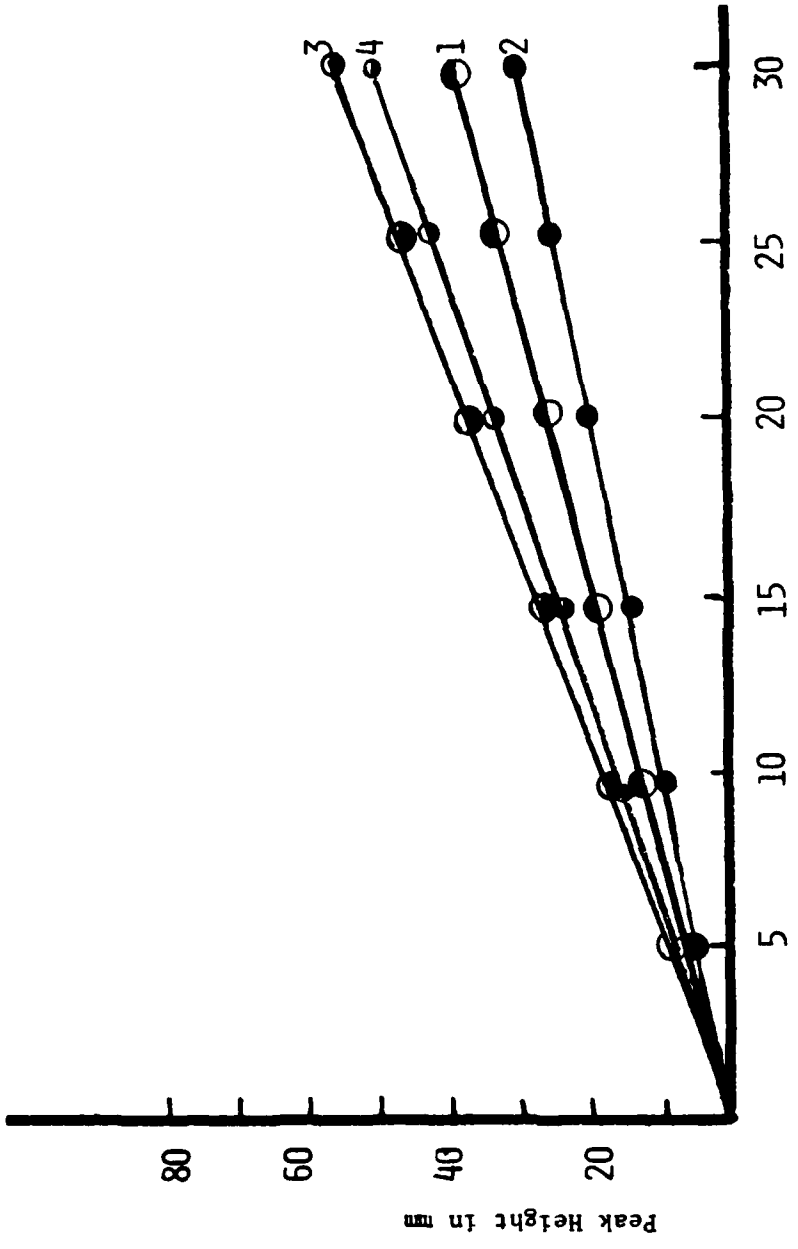


Fig. 2. Standard curves using HPLC elution systems A (compound 1, 2 and 3) and system B (compound 4). See legend Fig. 1-A and 1-B for code.

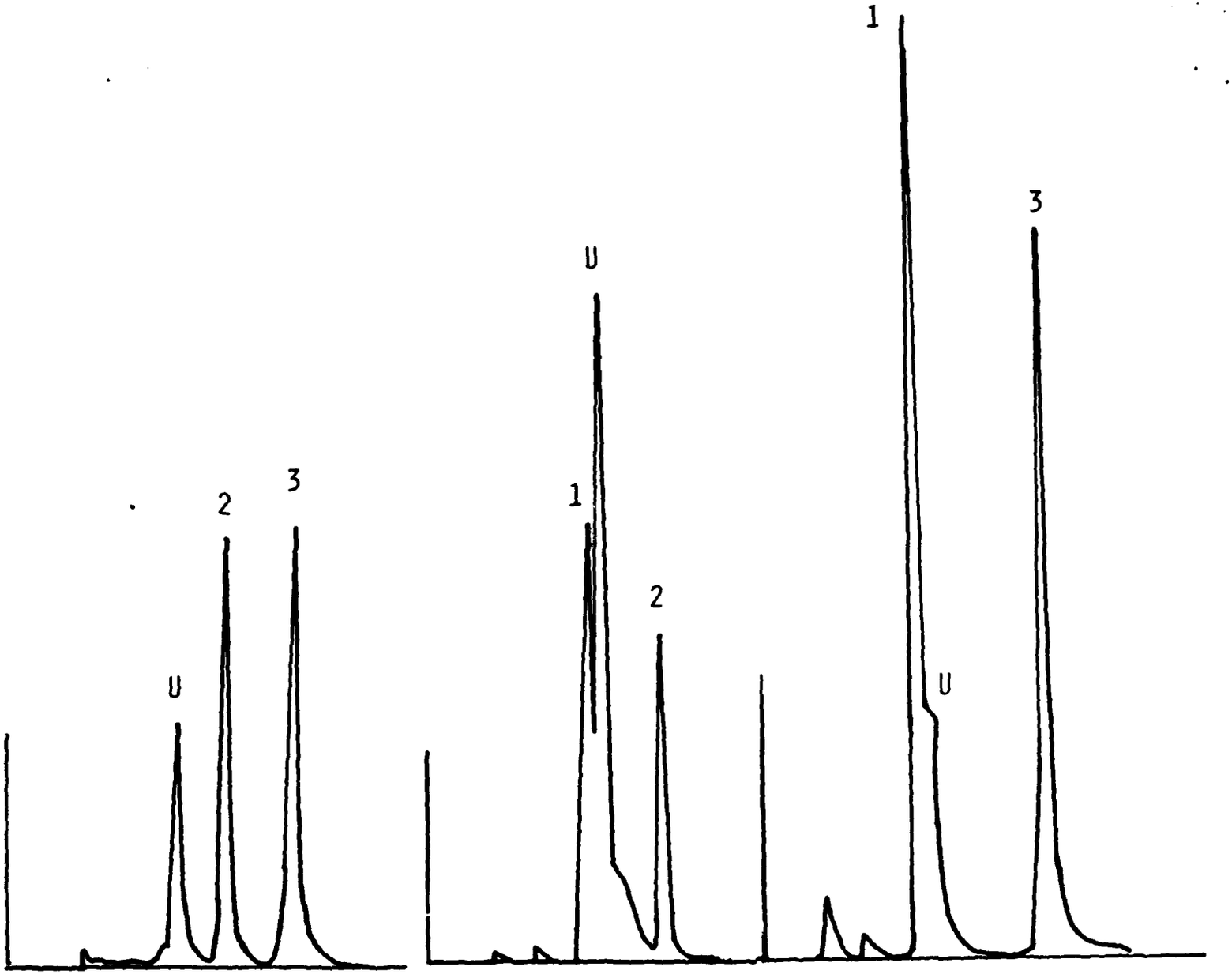


Fig. 3. Extraction mixtures chromatographed using elution System A. showing Unknown (U) compound probably formed by redox interaction.

previously screened for aceptability on the basis of their reponse to an interdermal injection of purified trichopyton antigen (Hollister Steer Company) to assess their immune reaction. The subjects were selected who showed immediate reaction, indicating they had developed an immune response to the antigen. Subjects with a delayed reaction to the antigen were not included in the study. Experiments were conducted using four sites, two each on the upper and lower arms of volunteers (See Figure 4). The areas were isolated by tape strips. Approximately 100 mg of the lotion vehicles were applied twice daily to randomly selected one of four sites on the forearm of the volunteers using the lotion containing 0.0,0.2,0.5 and 1.0% concentrations of sodium pyrithione. The volunteers were allowed to wash with water during the intervening period,but not with soap or detergents. The applications were continued for three days. Twenty four hours after the last appliation, the four areas on both arms were infected with application of 600 fungal spores of T. Mentagrophytes which had been standardized by Dr. Joe Greenberg.

Two groups of 8 subjects were scheduled for the studies. Three subjects dropped out of the first study and two from the second all for extra-medical reasons. A total of 11 completed the study. After four days of occlusion the saran plastic wrap was removed from the sites. Subjects were seen on a weekly basis by Dr. Epstein and individually scored. As to the severity of the reaction Dr. Epstein examined the volunteers without knowledge of the location of the individual treatment. He scored the response as noted below:

0 = Normal skin, slightly scaled without erythema.

+1 = Less than 6-10 tiny erythematous follicular papules or 1-2 larger erythematous papules.

+2 = More than 10 papules or pustules, colonly formation or coalescence. (Figure 5 is a copy of the scoring Form)

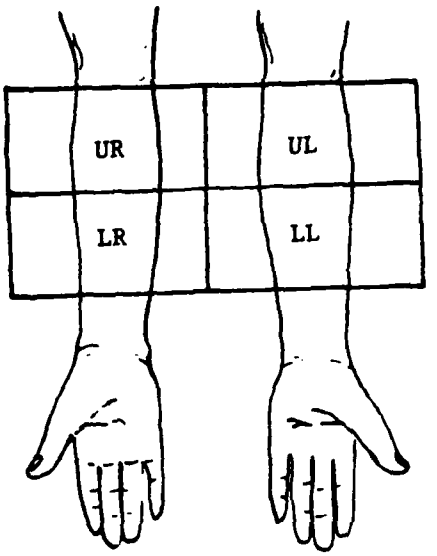
As per our original investigations on other anti-fungal drugs, the score selected for the intersubject comparison is based on the time when the infection reached maximum for each individual. This decision was made prior to breaking the code. Table II represents the data group according to drug concentrations applied. The sum of the scores for each subject is listed at the bottom of each column. There are respectively.

Placebo	15
0.2%	9
0.5	3
1.0%	2

FIG. 4

INFECTIVITY STUDIES

SITES OF APPLICATION



UR: Upper portion of the Right Arm

LR: Lower portion of the Right Arm

UL: Upper portion of the Left Arm

LL: Lower portion of the Left Arm

FIG. 5

SUBJECT .....

SCORING OF INFECTION

EXPERIMENT ..... GROUP .....

DATE	RU	RL	LU	LL

0 - normal skin, slight scaling without erythema
+1 - < 6-10 tiny erythematous follicular papules or 1-2 larger erythematous papules
+2 - > 10 papules or pustules, colony formation or coalescence

TABLE II

DOSE RANGING INFECTIVITY TEST DATA

RESPONSE GROUPED ACCORDING TO PERCENT PYRITHIONE

CONCENTRATION APPLIED TO TEST SITE

SUBJECT	PLACEBO	0.2%	0.5%	1.0%
1	2	2	0	0
2	1	0	0	0
3	1	0	0	0
4	1	0	0	0
5	1	0	1	0
6	1	0	0	0
8	1	1	0	0
10	2	0	1	1
11	2	2	0	0
12	1	2	1	1
14	2	2	0	0
	15	9	3	2

Without attempting statistical evaluation of these data, it is clear that these values point to marked difference in the therapeutic effectiveness. Table III presents the values grouped according to the site of application on the forearm. The scores at each of the four sites are summarized at the bottom of each column. They are:

RU = 5

RL = 7

LU = 10

LL = 7

It may be concluded, from these results that there is virtually no difference in sensitivity to infections at the various sites.

As per our earlier analyses, we utilized a Chi-square Contingency Table Analysis using a Fisher Exact Test. Chi-square analysis of the total data is shown in Table IV and shows a significant difference between placebo and test lotions used in the overall study ($P<0.001$). The data were therefore substratified to test for comparative significance among different combinations. The chi-square analysis of placebo vs. 0.2% is summarized in Table V. These results indicate a statistically significant difference between the placebo and the 0.2% lotion ($P< 0.001$)

In Table VI is a chi-square analysis of 0.2% vs. 0.5 lotions, which was found to be non-significant ($0.1<P<0.5$). Comparison of 0.5% to 1.0% in Table VII also led to results that these two preparations were not statistically different as viewed the test results. Finally, comparison of the 0.2% to the 1.0% led to data that was found to be non-significant (Table VIII).

Based on the results of this concentration-dose-ranging experiment, we conclude that all preparations are statistically significantly different from the placebo and show activity under the test procedure. It is particularly noted that the most dilute concentration tested, i.e.,0.2% sodium pyrithione was highly active. Since the latter tested at $P<0.001$, it is likely that even a lower concentration would have shown activity if tested.

Relative to selection of the concentration to be tested in further studies comments obtained from the volunteers on the study indicated that there was a slight local irritation at the site of application of the 1% lotion. Therefore, it was decided that we would continue the study utilizing 0.5% lotion.

Sodium Pyrithione Persistence - Infectivity Study

This experiment was designed in order to assess how long after application

TABLE III

DOSE RANGING INFECTIVITY TEST DATA

RESPONSE GROUPED ACCORDING TO THE SITE OF APPLICATION

SUBJECT	RU	RL	LU	LL
1	0	2	0	2
2	0	0	1	0
3	0	1	0	0
4	1	0	0	0
5	0	0	1	1
6	0	0	1	0
8	1	0	1	0
10	0	1	2	1
11	0	2	0	2
12	1	1	2	1
14	2	0	2	0
	5	7	10	7

TABLE IV

DOSING RANGING - INFECTIVITY TEST

FISHER EXACT TEST OF TOTAL DATA

		% APPLIED TO SITE				
χ^2-Analysis	Response	Placebo	0.2	0.5	1.0%	Totals
E= (5.750)	0	0	6	8	2	23
$(O-E)^2/E$ =		5.750	.011	0.880	1.237	
E=(3.250)	1+	7	1	3	2	13
$(O-E)^2/E$ =		4.327	1.558	0.019	0.481	
E= (2.000)	2+	4	4	0	0	8
$(O-E)^2/E$ =		2.0	2.0	2.0	2.0	
Totals		11	11	11	11	44

$\chi^2 = \sum_1^n \frac{(O-E)^2}{E} = 22.863$

d.f = (3-1) (4-1) = 6

χ^2 0.05,6 = 12.592

χ^2 0.001,6 = 22.458 P< 0.001

TABLE V

DOSE RANGING -INFECTIVITY TEST

FISHER EXACT TEST OF PLACEBO VS. 0.2%

% APPLIED TO SITE

χ^2-Analysis	Response	Placebo	0.2%	Totals
E= (3.000)	0+	0	6	6
$(O-E)^2/E$ =		3.000	3.000	
E =(4.000)	1+	7	1	8
$(O-E)^2/E$=		2.250	2.250	
E =(4.000)	2+	4	4	8
$(O-E)^2/E$=		4.000	4.000	
Totals		11	11	22

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 18.500$$

d.f. = (2-1) (3-1)= 2

χ^2 0.05,2 = 5.991

χ^2 0.001,2 = 10.828 P< 0.001

TABLE VI

DOSE RANGING INFECTIVITY TEST

FISHER EXACT TEST OF 0.2% vs. 0.5%

% APPLIED TO SITE

χ^2-Analysis	Response	0.2	0.5	Totals
E = (7.000)	0	6	8	14
$(O-E)^2/E$ =		0.143	0.143	
E = (2.000)	1+	1	3	4
$(O-E)^2/E$=		0.500	0.500	
E = (2.000)	2+	4	0	4
$(O-E)^2/E$=		2.000	2.000	
Totals		11	11	22

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 5.286$$

d.f. = (2-1) (3-1)= 2 P = 0.064

χ^2 0.05,2 = 5.991 N.S.

TABLE VII

DOSE RANGING INFECTIVITY TEST
FISHER EXACT TEST OF 0.5% VS. 1.0%

% APPLIED TO SITE

χ^2-Analysis	Response	0.5	1.0	Totals
E = (8.5)	0	8	9	17
$(O-E)^2/E$ =		0.029	0.029	
E = (2.5)	1+	3	2	5
$(O-E)^2/E$=		0.100	0.100	
E = (0)	2+	0	0	0
$(O-E)^2/E$=		0	0	
Totals		11	11	22

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 0.259$$

$\chi^2_{0.05,2} = 5.991$

$\chi^2_{0.90,2} = 0.211$ $\qquad$ $0.9 < P < 0.75$

$\chi^2_{0.75,2} = 0.575$

TABLE VIII

DOSE-RANGING - INFECTIVITY TEST

FISHER EXACT TEST OF 0.2 VS. 1.0%

χ^2-Analysis	Response	% APPLIED TO SITE 0.2	1.0	Totals
E = (7.500)	0	6	9	15
$(O-E)^2/E$ =		.300	.300	
E = (1.500)	1+	1	4	3
$(O-E)^2/E$=		.167	.167	
E = (2.00)	2+	4	0	4
$(O-E)^2/E$=		2.000	2.000	
Totals		11	11	22

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 4.933$$

d.f. = (2-1) (3-1)= 2

χ^2 0.05,2 = 5.991 0.05 P< 0.1

χ^2 0.1,2 = 4.605 .

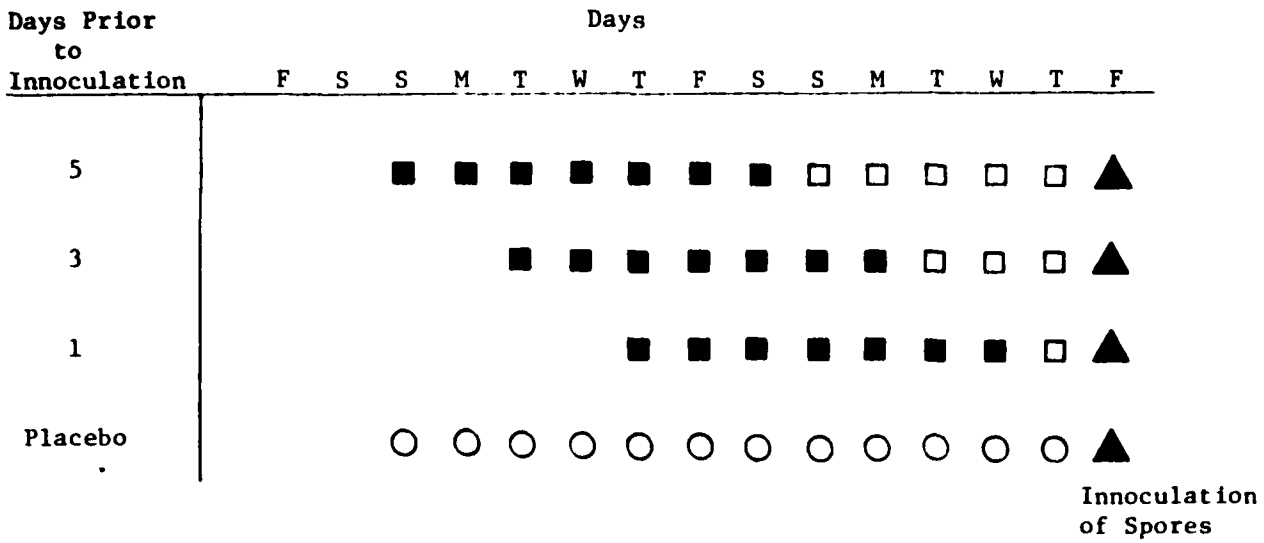
was discontinued did sodium pyrithione retain prophylactic activity. The study was designed to address the question: After application of the drug for five days (to reach equilibrium of the pyrithione in the horny layer of the skin), how many days after application was stopped does the residual sodium pyrithione remaining in the tissues still maintain anti-fungal activity? This design was adopted since soldiers may be in active combat for several days under conditions where they are unable to renew the application. This study was performed on two groups of test subjects. Twelve subjects completed to total trial. The protocol design is summarized in Figures 6 and 7. The 0.5% sodium pyrithione lotion prepared by G. S. Herbert Labs was applied randomly on three of the four sites on the forearm. The fourth site remained a placebo site (Figure 4). The solution was applied for seven days to each site (Figure 6). However, the initial applications were staggered such that at the end of the application period 1,3, or 5 day occurred prior to the innoculation of spores, (See Figure 7). The volunteers were allowed to use normal hygiene. They were, however, encouraged not to apply to the areas during the application period At the appropriate time after the last application, the three sites and placebo sites were infected with 600 spores of T. Menta. The areas were covered with gauze dressing and occluded with Saran Wrap for four days, after which the bandages were taken off. The sites on the arms were examined by Dr. Epstein weekly for the next several weeks until the infection subsided. If any infections were not cured spontaneously by four weeks beyond the end of the trial, griseofulvin was administered (500 mg bid) until the infections were eliminated. As earlier, the infections were scored as 0, +1, and +2 using the scoring form (Figure 4). The scoring was done on a double-blind basis and before breaking the code, a week was chosen on which produced the greatest degree of response. Table IX includes a summary of the data from 12 subjects who completed the trial. The bottom of each column is a summary of the total number of scores for the number of days post application prior to spore innoculation. These are summarized below.

DAYS POST APPLICATION	TOTAL
Placebo	17
5	14
3	9
1	4

From the results summarized above it can be seen that five days application produced results nearly identical to the placebo site and one to three days apparently afforded significant difference in score values relative to the placebo site.

FIG. 6

Sodium Pyrithione Persistance Study Schedule



■ Application of griseofulvin twice daily

□ No application

○ Placebo site

Note: Lotion is only applied to three sites - one site is left as a control.

FIG. 7

STUDY PLAN SODIUM PYRITHIONE PERSISTENCE STUDY

Weeks of Study

Dates:	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	M	F	M	M	M
Sub.#							(*)																	
					LU	LU	LU	LU	LU	LU	LU	0	0	0	I				P	R		R	R	R
							LL	LL	LL	LL	LL	LL	LL	0	I				P	R		R	R	R
	RU	RU	RU	RU	RU	RU	RU	0	0	0	0	0	0	0	I				P	R		R	R	R
			RL	RL	RL	RL	RL	RL	RL	0	0	0	0	0	I				P	R		R	R	R

0 - No application

I - Infection, 600 spores T. Mentogrophytes

P - Removal of Patches

R - Readings

* Note: Sides are randomized for each test subject.

Arm Site

LU - Left Upper

LL - Left Lower

RU - Right Upper

RL - Right Lower

Table X presents the data according to the site of application on the forearm. The scores agree from each of the four sites (See Figure 3) are summarized at the bottom of each column. These are:

RU - 11

RL - 8

LU - 12

LL - 13

It may be concluded therefore that there are virtually no differences in sensitivity to infection at the various sites.

Therefore, a chi-square analysis of the total data, was performed and is summarized in Table XI. The results indicate that there is a statistically significant difference in the trial groups ($0.01<P<0.023$), and substratification analyses were undertaken. Table XII includes a summary of comparison of Placebo vs. day-1. From this table is to be seen that there is a statistically significant difference in resistence to infectivity 24 hours after the application of sodium pyrithione had ceased ($P<0.001$). This once again confirms the results obtained in the dose ranging study. Table XIII summarized the comparison of placebo vs. day-3. These results show relative to the placebo site, is statistically significant resistence of infection 72 hours after application of sodium pyrithione had ceased ($0.01<P<0.025$)contrasted placebo.

Table XIV includes a comparison of placebo vs. day-5, and indicates there is no statistically significance between the results ($0.25<P<0.50$). This indicates that the residual drug concentration after five days is not sufficient to produce the resistence to infection when compared to the placebo site under the test conditions. Table XV and XVI make comparisons of day-3 vs. day-5 and day-1 vs. Day respectively. From these results, it is apparent that there is a statistically significant difference between day-1 and day-5 ($0.01<P<0.020$)while there is not significant difference between days-3 and 5 ($0.1<P<0.25$).

TABLE IX

PERSISTENCE-INFECTIVITY DATA

RESPONSE GROUPED ACCORDING TO DAYS AFTER LAST APPLICATION

SUBJECT	PLACEBO	1	3	5
1	2	0	2	2
2	2	0	0	1
3	1	1	0	1
4	1	0	2	2
5	1	0	0	1
6	1	1	1	0
7	2	0	0	0
8	1	0	2	2
9	1	1	0	1
10	2	1	1	2
11	1	0	0	1
12	2	0	1	1
	17	4	9	14

TABLE X

PERSISTENCE-INFECTIVITY TEST DATA

RESPONSE GROUP ACCORDING TO SITE OF APPLICATION

SUB	RU	RL	LU	LL
1	2	0	2	2
2	2	0	0	1
3	1	0	1	1
4	2	0	1	2
5	0	1	1	0
6	1	0	1	1
7	0	0	0	2
8	1	2	2	0
9	0	1	1	1
10	2	2	1	1
11	0	0	1	1
12	0	2	1	1
	11	8	12	13

TABLE XI

PERSISTENCE INFECTIVITY TEST

FISHER EXACT TEST OF TOTAL DATA

DAYS POST APPLICATION

X^2-Analysis	Response	Placebo	1	3	5	Totals
E= (4.000)	0+	0	8	6	2	16
$(O-E)^2/E$ =		4.000	4.000	2.667	.167	
E=(5.000)	1+	7	4	3	6	20
$(O-E)^2/E$ =		0.800	.200	.800	.200	
E= (3.000)	2+	5	0	3	4	12
$(O-E)^2/E$ =		1.333	3.000	0	.333	
Totals		12	12	12	12	48

$$X^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 14.833$$

d.f = (3-1) (4-1) = 6

X^2 0.05,6 = 12.593 0.01<P<0.0125

X^2 0.025,6 = 14.449

X^2 0.01,6 = 16.812

TABLE XII

PERSISTENCE INFECTIVITY TEST DATA

FISHER EXACT TEST OF PLACEBO VS. DAY-ONE

DAYS POST APPLICATION

χ^2-Analysis	Response	Placebo	1	Totals
E= (4.00)	0+	0	8	8
$(O-E)^2/E$ =		4.000	4.000	
E =(5.500)	1+	7	4	11
$(O-E)^2/E$=		0.409	0.409	
E =(2.500)	2+	5	0	5
$(O-E)^2/E$=		2.500	2.500	
Totals		12	12	24

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 13.313$$

d.f. = (2-1) (3-1)= 2

χ^2 0.05,2 = 5.991

χ^2 0.001,2 =13.816 P= 0.001

TABLE XIII

PERSISTENCE INFECTIVITY TEST

FISHER EXACT TEST OF PLACEBO VS. DAY-TIME

DAYS POST APPLICATION

X^2-Analysis	Response	Placebo	Day 3	Totals
E = (3.00)	0	0	6	6
$(O-E)^2/E$ =		3.00	3.00	
E = (5.000)	1+	7	3	10
$(O-E)^2/E$=		0.800	0.800	
E = (4.000)	2+	5	3	8
$(O-E)^2/E$=		.250	.250	
Totals		12	12	24

$$X^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 8.100$$

d.f. = (2-1) (3-1)= 2

X^2 0.05,2 = 5.991

X^2 0.025 = 7.378 $\quad 0.01 < P < 0.025$

X^2 0.01 = 9.21

TABLE XIV

PERSISTENCE INFECTIVITY TEST

FISHER EXACT TEST OF PLACEBO VS. DAY-FIVE

DAYS POST APPLICATION

χ^2-Analysis	Response	Placebo	Day 5	Totals
E = (1.00)	0	0	2	2
$(O-E)^2/E$ =		1.000	1.000	
E = (6.500)	1+	7	6	13
$(O-E)^2/E$=		0.038	0.038	
E = (4.500)	2+	5	4	9
$(O-E)^2/E$=		0.056	0.056	
Totals		12	12	24

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 2.188$$

d.f. = (2-1) (3-1)= 2

χ^2 0.05,2 = 5.991

χ^2 0.25,2 = 2.773 0.25<P<0.50

χ^2 0.5 ,2 = 1.386 NS

TABLE XV

PERSISTENCE INFECTIVITY TEST

FISHER EXACT TEST OF DAY ONE VS. DAY FIVE

DAYS POST-APPLICATION

χ^2-Analysis	Response	1	5	Totals
E = (5.000)	0	8	2	10
$(O-E)^2/E$ =		1.800	1.800	
E = (5.000)	1+	4	6	10
$(O-E)^2/E$=		.200	.200	
E = (2.000)	2+	0	4	4
$(O-E)^2/E$=		2.000	2.000	
Totals		12	12	24

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 8.000$$

d.f. = (2-1) (3-1)= 2

χ^2 0.05,2 = 5.991

χ^2 0.025,2 = 7.378 .01<P < 0.02

χ^2 0.0.10,2 = 9.210

TABLE XVI

PERSISTENCE-INFECTIVITY TEST

FISHER EXACT TEST OF DAY-THREE VS. DAY-FIVE

DAYS AFTER APPLICATION

χ^2-Analysis	Response	3	5	Totals
E= (4.00)	0	6	2	8
$(O-E)^2/E$ =		1.000	1.000	
E = (4.500)	1+	3	6	9
$(O-E)^2/E$=		0.500	0.500	
E = (3.500)	2+	3	4	7
$(O-E)^2/E$=		0.07	0.07	
Totals		12	12	24

$$\chi^2 = \sum_{1}^{n} \frac{(O-E)^2}{E} = 3.14$$

d.f. = (2-1) (3-1) = 2

$\chi^2_{0.05,2} = 5.991$

$\chi^2_{0.1,2} = 4.605$ $\quad 0.10<P<0.25$

$\chi^2_{0.25} = 2.776$

Summary

Based on the above statistical analysis of the persistence infectivity trial, one can conclude that after application of sodium pyrithione 0.5% lotion (G.S. Herbert Labs) for seven days to achieve steady state, that residual concentrations of sodium pyrithione persists at sufficient concentration to the horny layer at least three days after application has ceased to maintain prophylactic activity under the controlled infectivity test procedure. These results are highly encouraging since they indeed appear to exceed somewhat that which was obtained with griseofulvin. In earlier studies utilzing a similar protocol wherein griseofulvin was applied in a 0.45% concentration in ethanol, in a similar persistence - infectivity trial (4). Comparison of the placebo site to the three day, five day and seven day sites of 21 subjects who completed the trial, indicated there was no significant difference between day-zero and day-three (See Table XVII).

Sodium pyrithione in a 0.5% lotion as formulated by G. S. Herbert laboratories appears to retain topical activity at 72 hours after application has ceased while griseofulvin failed the similar test. We therefore recommend that that the sodium pyrithione 0.5% lotion warrants serious consideration as the alternative to griseofulvin for prophylactic use when and if needed to combat T. Menta infections.

Table XVII

Magnitude of Topical Infection Following Application of 450 T. menta Spores 1, 3, 5 and 7 days after the Last Topical Application of Griseofulvin

Subject	Placebo	Days between the last Griseofulvin application and infection		
		3	5	7
1.	++	0	0	0
2.	++	0	0	0
3.	++	++	++	++
4.	++	0	++	++
5.	++	++	+	+
6.	++	++	++	++
7.	++	++	++	0
8.	++	++	+	++
9.	++	++	+	++
10.	++	0	0	++
11.	++	++	0	0
Total	22	14	11	13
12.	+	0	++	++
13.	+	++	++	+
14.	+	+	++	+
15.	+	++	+	+
16.	++	+	+	++
17.	+	0	0	+
18.	+	++	++	++
19.	++	++	++	0
20.	++	++	0	++
21.	++	+	+	+
Total	14	13	13	13
Overall Total	36	27	24	26

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